

**REMARKS/ARGUMENTS**

This application is a division of earlier application Serial No. 10/093,508 filed March 11, 2002. See the first Official Action, Paper No. 4 dated February 20, 2003 (copy attached) and the requirement for restriction presented in it. The claims presented herein are directed to methods for analyzing cations by ion chromatography using specified eluents.

Attention is invited to the documents cited during the examination of the parent application (where copies may be found) and to the concurrently filed Information Disclosure Statement to the extent these documents may be relevant to the above method claims.

Applicants' claims are directed to a method for analyzing cations by ion chromatography using a specific eluent. The eluent contains an acid preferably selected from nitric acid, sulfuric acid, phosphoric acid, methanesulfonic acid, oxalic acid, tartaric acid, benzoic acid and phthalic acid, and at least one amino acid selected from the group consisting of histidine, lysine and arginine.

The object of the present invention is, as described on page 3, lines 4-26 of applicants' specification, to employ an eluent (1) which is capable of permitting an alkaline earth metal such as magnesium or calcium to elute in a good peak shape (absence of tailing) and (2) which is capable of not permitting a system peak, that is a peak not attributable to cations in the test samples to appear or overlap hence avoiding an influence over the analysis of cations.

As described on page 11, lines 13-22, the eluent used in the method of the present invention has the following effects:

- (1) It is possible to elute alkaline earth metals such as magnesium and calcium in good peak shapes; and
- (2) It is possible to avoid the appearance of a system peak that otherwise produces an unwanted influence over the analysis.

These effects are evident from the Examples and comparative Examples included in the specification. As an illustration, the eluents used in Example 1 (from page 8, line 17 to page 9, line 13) and Comparative Example 1 (page 10, lines 7-14) both contain nitric acid but they are different only in that the eluent in Example 1 also contains histidine while the eluent used in Comparative Example 1 does not. Applicants' claims require the use of at least one of the amino acids specified of which histidine is included.

In Example 1, magnesium ions and calcium ions were eluted in good peak shapes and gave sharp peaks designated as peaks 5 and 6, respectively, as shown in Fig. 1. The asymmetry factor of 1.55 for peak 6 attributable to calcium ions (page 9, lines 7-13) also indicates that calcium ions were eluted in a good peak shape.

On the other hand, in Comparative Example 1, both magnesium ions and calcium ions were eluted in unfavorable peak shapes and gave tailing peaks 5 and 6, respectively, as shown in Fig. 4. Especially, calcium ions gave a severely tailing peak (peak 6) with an asymmetry factor of 5.92 (page 10, lines 12-14).

The difference in the peak shapes is attributable to the difference in the eluent. Thus, the combined use of nitric acid, sulfuric acid, phosphoric acid, methanesulfonic acid, oxalic acid, tartaric acid, benzoic acid or phthalic acid, in combination with at least one amino acid selected from histidine, lysine and arginine as defined in applicants' claims produces excellent effects.

Hafeli (U.S. 4,259,447) cited during the examination of the parent application relates to purification of urokinase by liquid chromatography using an eluent which may contain acetic acid or HCl (column 3, line 42-43), and amino acids such as lysine and arginine (column 3, lines 45-47). It will be apparent Hafeli does not disclose any of the claimed specific acids or the claimed combination of a specific acid and a specific amino acid. Further, Hafeli is totally irrelevant to analysis of alkaline earth metals. Because the object of Hafeli is purification of urokinase, unlike that of the present invention, Hafeli naturally is silent about the above-mentioned positive effects achieved by the method of the present invention.

Heimburger (US 3,732,146) also cited during the examination of the parent application relates to isolation of plasminogen by liquid chromatography using an eluent containing hydrochloric acid and lysine. Heimburger does not disclose any of the claimed specific acids or the claimed combination of a specific acid and a specific amino acid. Further, Heimburger is totally irrelevant to analysis of alkaline earth metals. Thus, because the object of Heimburger (isolation of plasminogen) is different from that of the present invention, Heimburger is naturally silent about the above-mentioned positive, unexpected effects of the present invention.

Hajos (Journal of Chromatography, 1997) also cited during the examination of the parent application relates to separation of cations such as magnesium and calcium ions by liquid chromatography using acidified histidine as an eluent and discloses an eluent containing HCl and histidine as an example. Hajos does not specifically disclose any acids other than HCl. Thus, Hajos does not disclose any of the claimed specific acids or the claimed combination of a specific acid and a specific amino acid. In addition to the fact that Hajos does not mention the unexpected effects of the present invention (i.e., it is possible to elute alkaline earth metals such as magnesium and calcium in good peak shapes), the eluent disclosed in Hajos, on the contrary, produced worse results and magnesium ions and calcium ions were eluted in unfavorable peak shapes as is evident from the tailing of the peaks attributable to magnesium ions and calcium shown in Figs. 2, 4, 5 and 6.

Japan 06-018505 also cited during the examination of the parent application relates to analysis of cations such as magnesium ions and calcium ions using a dilute phosphoric acid aqueous solution as an eluent. However, Japan 06-018505 does not suggest incorporation of an amino acid in the eluent at all, not to mention the use of the three specifically claimed amino acids. Further, Japan 06-018505 does not disclose the claimed combination of a specific acid and a specific amino acid. Japan 06-018505 is also silent about the unexpected effects achieved by the process of the present invention (that it is possible to elute alkaline earth metals such as magnesium and calcium in good

peak shapes). On the contrary, with the eluent disclosed in Japan 06-018505, magnesium ions and calcium ions were eluted in unfavorable peak shapes as is evident from the tailing of peaks 5 and 6 in Figs. 2 and 3, and thus unfavorable results were obtained.

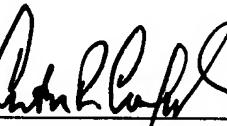
Japan 08-257419 also cited during the examination of the parent application relates to analysis of cations such as magnesium ions and calcium ions by liquid chromatography using an eluent containing an acid such as nitric acid. Japan 08-257419 does not suggest incorporation of an amino acid in the eluent at all, not to mention the use of any of the three specifically claimed amino acids. Further, Japan 08-257419 does not disclose the use of the claimed combination of a specific acid and a specific amino acid. Japan 08-257419 is also silent about the effect of the present invention (that it is possible to elute alkaline earth metals such as magnesium and calcium in good peak shapes). On the contrary, with the eluent disclosed in Japan 08-257419, magnesium ions and calcium ions were eluted in unfavorable peak shapes as is evident from the tailing of peaks attributable to magnesium ions and calcium ions in Figs. 4, 7 and 8, and thus unfavorable results were obtained.

An examination on the merits is awaited.

Respectfully submitted,

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